Pilot Study of the air mycobiota of a cheese factory

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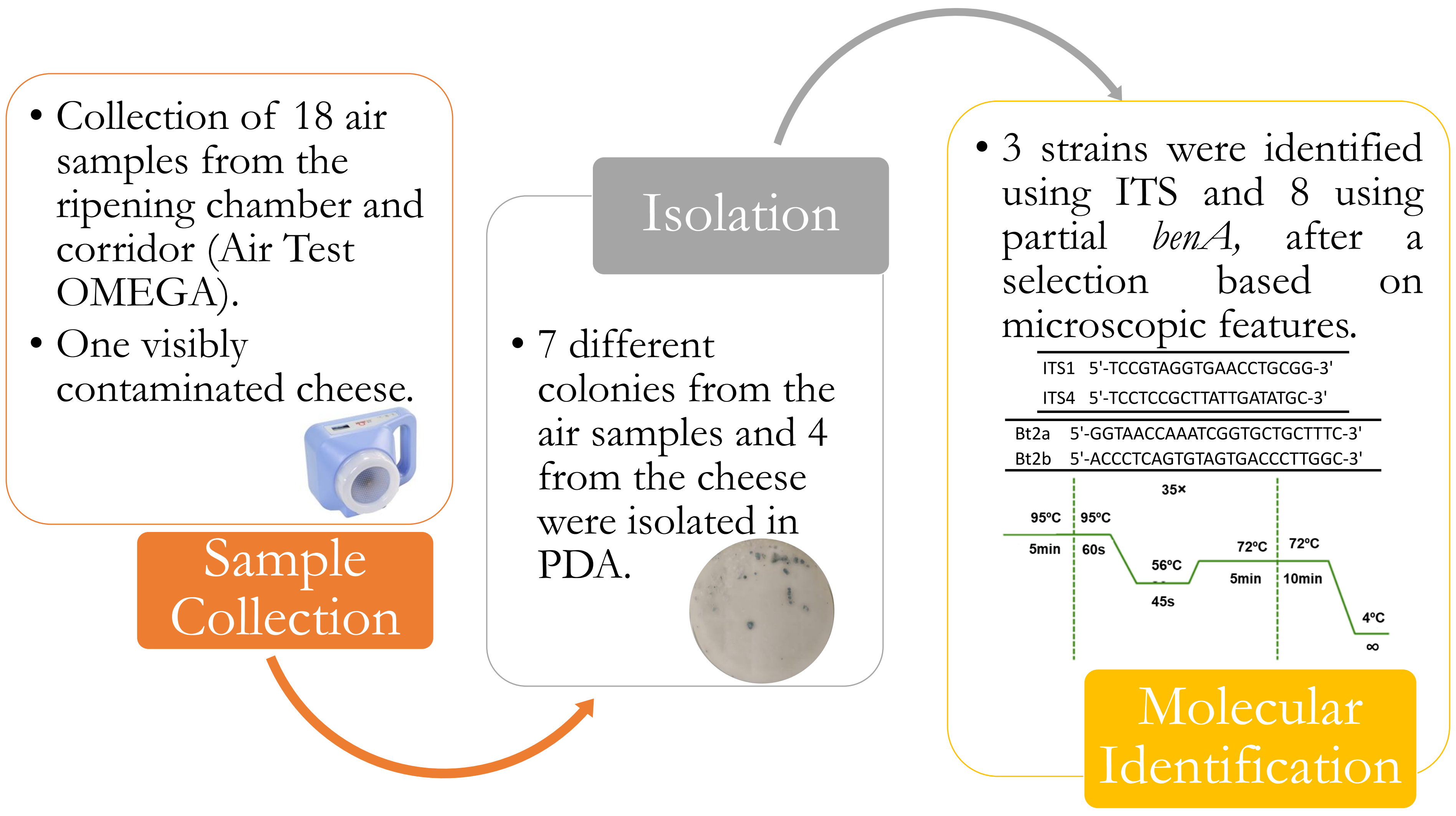
Introduction

Cheese is the common name given to fermented milk products. In essence, it is a concentration process, when the principal proteins of milk (caseins) coagulate, form a gel and entrap fat[1].

Some fungi aid the cheese production process, having a beneficial role. However, indigenous moulds of the ripening chambers may colonize the surface of cheeses, as conditions at which ripening occurs are favourable to fungi development. This opportunistic uncontrolled colonization makes the product undesirable and can even present a health risk due to the production of secondary metabolites, such as mycotoxins[2].

This work intended to investigate the air mycobiota of a Portuguese cheese industry to uncover the role of the ripening chamber air as the source of the observed cheese contamination.

Material and Methods



Sample Collection

- Collection of 18 air samples from the ripening chamber and corridor (Air Test OMEGA).
- One visibly contaminated cheese.

Isolation

- 7 different colonies from the air samples and 4 from the cheese were isolated in PDA.

Molecular Identification

- 3 strains were identified using ITS and 8 using partial *benA*, after a selection based on microscopic features.

ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
ITS4	5'-TCCTCCGCTTATTGATATGC-3'
Bt2a	5'-GGTAACCAAATCGGTGCTGCTTTC-3'
Bt2b	5'-ACCCTCAGTGTAGTGACCCTTGGC-3'

Results

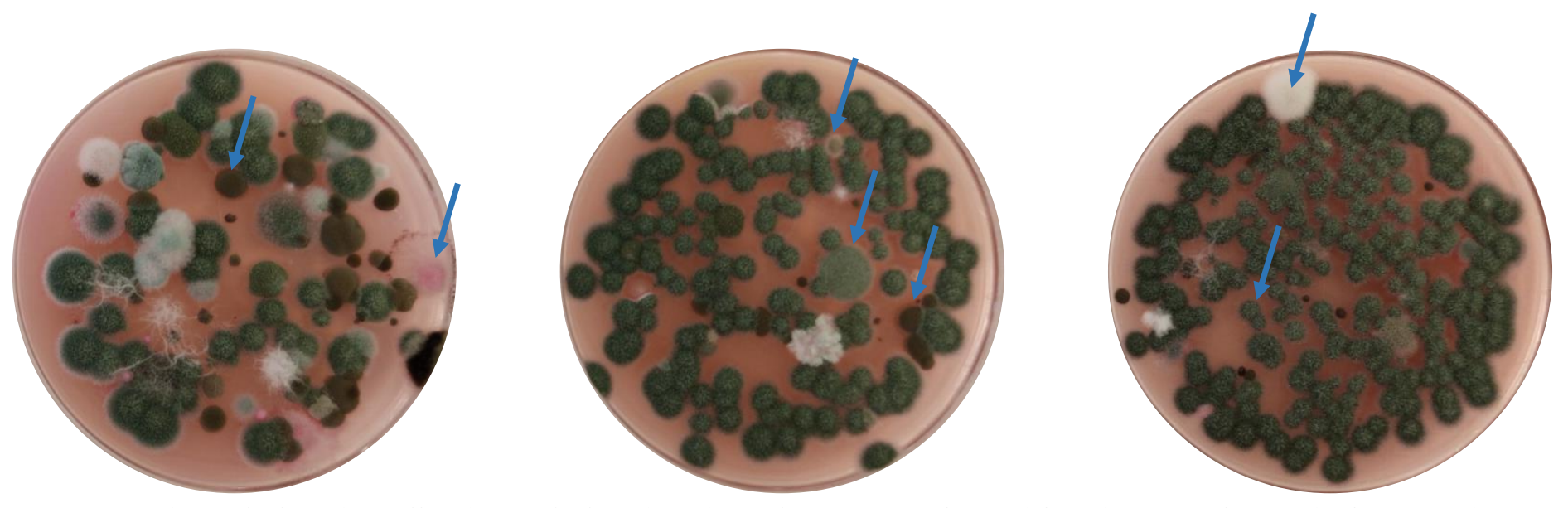


Fig. 1 – Examples of the air collection of the ripening chamber and corridor from a cheese industry, after 7 days growth at 25 °C. Air samples were collected using the impaction method on DRBC Petri dishes. The blue arrows point to the isolated colonies**.**

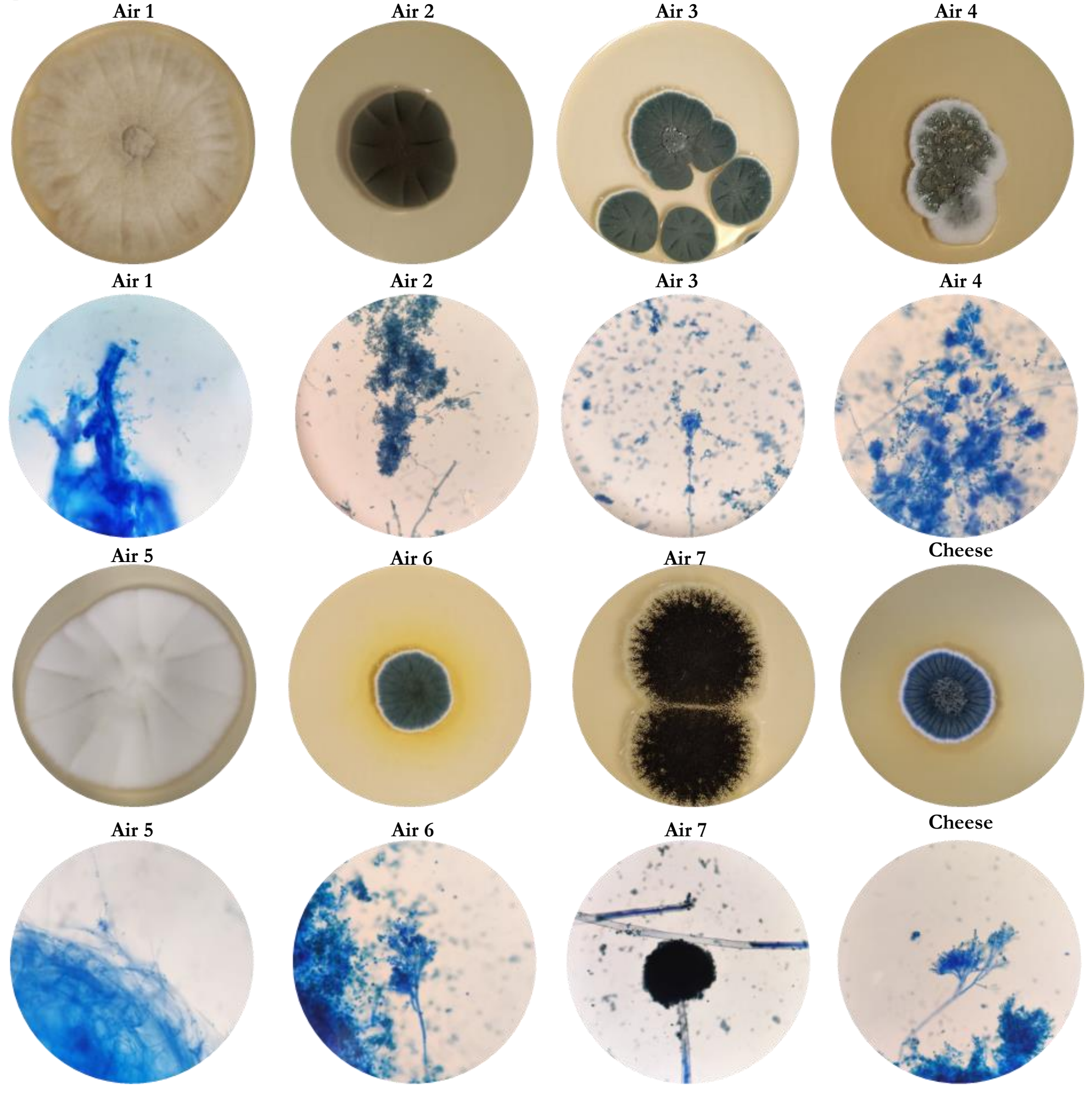


Fig. 2 – Macro and micro characterization of all the isolates from the air samples and one example from cheese, after 7 days growth in PDA at 25ºC.

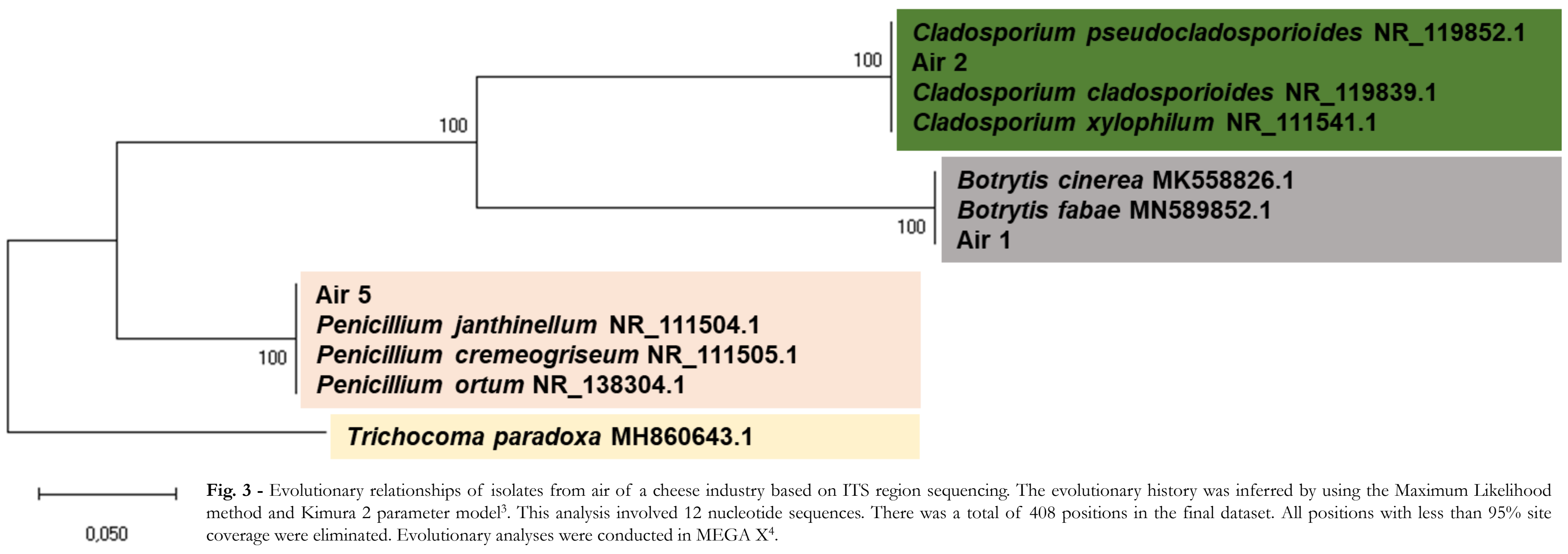


Fig. 3 - Evolutionary relationships of isolates from air of a cheese industry based on ITS region sequencing. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2 parameter model[3]. This analysis involved 12 nucleotide sequences. There was a total of 408 positions in the final dataset. All positions with less than 95% site coverage were eliminated. Evolutionary analyses were conducted in MEGA X[4].

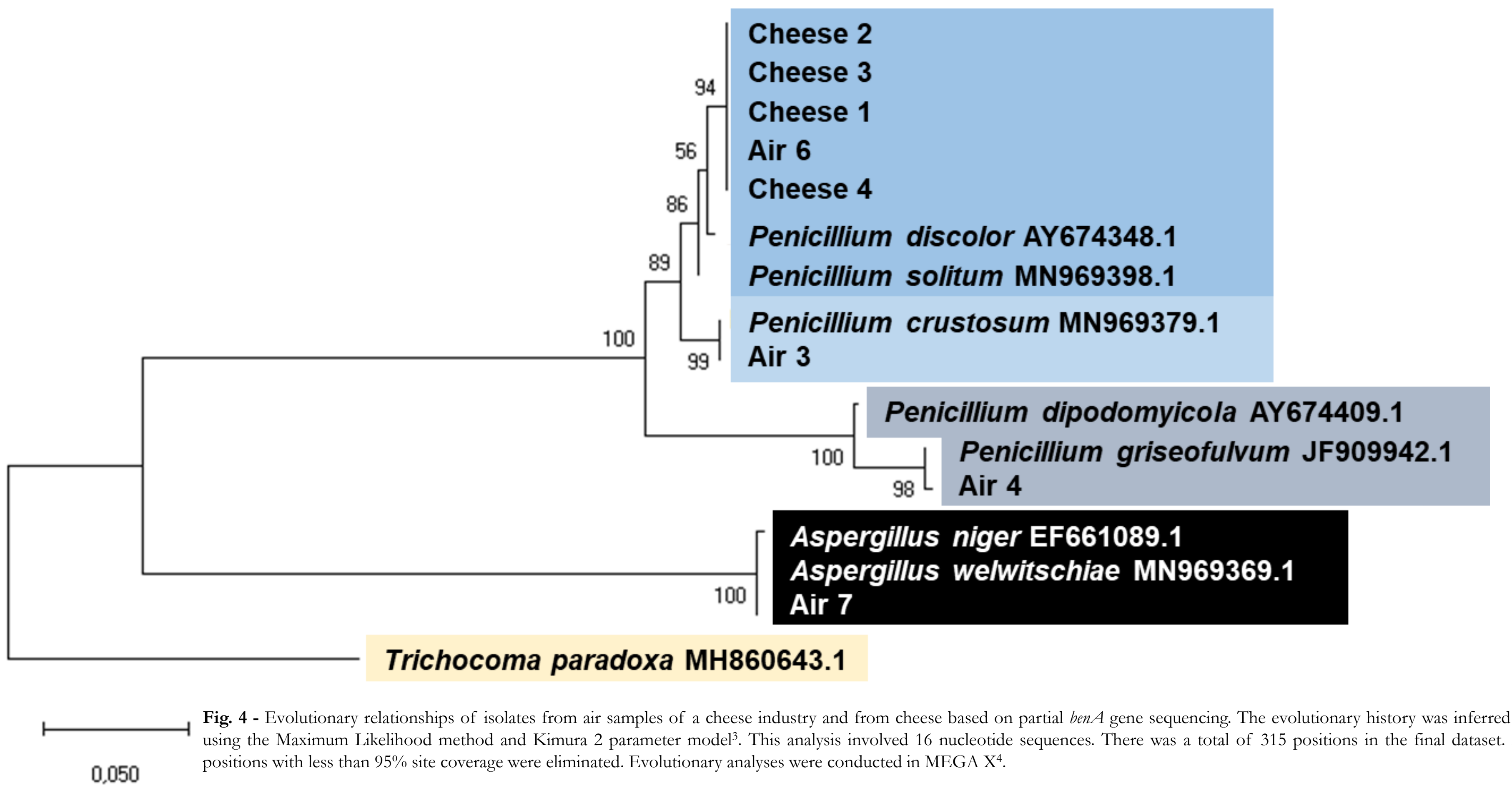


Fig. 4 - Evolutionary relationships of isolates from air samples of a cheese industry and from cheese based on partial *benA* gene sequencing. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2 parameter model[3]. This analysis involved 16 nucleotide sequences. There was a total of 315 positions in the final dataset. All positions with less than 95% site coverage were eliminated. Evolutionary analyses were conducted in MEGA X[4].

Discussion and Conclusion

This work is a preliminary study where colonies from a contaminated cheese were isolated as well as colonies from the air of the ripening chamber where the cheese was produced.

- Molecular identification was based on different molecular markers, depending on their preliminary morphological identification (Fig. 2). ITS is the universal barcode for the identification of fungi, however, beta-tubulin (*benA*) has been described as a more suitable marker for identification of *Penicillium* and *Aspergillus* to species level[5]. Consequently, the isolates that had microscopic features of *Penicillium* and *Aspergillus* were identified using *benA* and for the remaining isolates ITS was used.
- Four colonies were isolated from the cheese, all identified as *Penicillium* sp. (Fig. 4).
- From the air samples, seven isolates were obtained, belonging to *Penicillium*, *Botrytis*, *Cladosporium* and *Aspergillus* genera (Figs. 3, 4). Only two isolates were identified to species level, Air 3 – *Penicillium crustosum,* and Air 4 – *Penicillium griseofulvum*. These species have already been described as present in the air, surfaces and cheese rinds in other cheese facilities[6,7,8].
- Air 6 and all the isolates from the cheese clustered together (Fig. 4). Thus, in this case, cheese contamination seems to be due to the air of the ripening chamber, which is in agreement with previous studies[2]. Nevertheless, a fingerprint profile from the five strains is required to corroborate this theory.

In conclusion, this study indicates that, in this producing facility, the environment in the ripening chamber is a possible contamination source. Measures to mitigate this situation must be applied to reduce the cheese loss due to mould growth, as well as the potential health risk for the consumers, once fungi might produce secondary metabolites, such as mycotoxins.

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